

[COMMUNICATION]

Electron-Microscopic Observation of an Ectopic PGC-like Cell in the Teleost *Oryzias latipes*

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ABSTRACT—In one specimen of newly hatched fry of the medaka, *Oryzias latipes*, one primordial germ cell (PGC)-like cell was found by light microscopy in the subepidermal layer of the body wall lateral to the body cavity; an ectopic site far from the gonad where the normal primordial germ cells (PGCs) were actually located. The ultrastructures of the ectopic PGC-like cell was examined by electron microscopy, and compared with those of the normal PGCs in the gonad in the same specimen.

It was demonstrated that the above stated ectopic PGC-like cell, though spheroidal in shape, had striking morphological similarities without any degenerative aspect to the normal PGCs in the gonad at the ultrastructural level, especially due to the presence of two peculiar organella, i.e., the germinal dense bodies (GDB) and various lengths and forms of the sheets of agranular reticulum (AR).

The present evidence suggests that the ectopic PGC-like cell in the body wall might be one of the PGCs which had settled down in the aberrant site during migration into the gonadal region in the early stages of embryogenesis, and that the PGCs or their presumed forerunners, even in the ectopic site, could differentiate themselves at least to the same developmental stage as that of the normal PGCs actually located in the gonad at the time of hatching.

INTRODUCTION

In various animals including vertebrates and invertebrates, it has been fully confirmed that primordial germ cells (PGCs) segregate from the somatic cells during the early stages of development extra-gonadally or extra-embryonally, and

then migrate through various tissues towards their final location as the ultimate gametes in the gonadal anlagen [1]. The occurrence of the PGCs in various ectopic sites after the completion of their migratory journey into the gonadal anlagen has been reported in embryos of several species of vertebrates [2]. On the other hand, persistence and differentiation of the ectopic PGCs past the embryonic period have been reported only sporadically by Stolk [3], Hardisty [4], and Zamboni and Upadhyay [5]. Recently in the field of the study of germ cell sex differentiation in the medaka (*Oryzias latipes*) using light microscopy, we encountered with a fortunate opportunity to note the presence of one ectopic PGC-like cell within the cell layer constituting the lateral body wall, together with the normal PGCs in the gonad in the same plane of an epon-embedded section of one specimen of newly hatched fry. Thus far, ectopic PGCs or PGC-like cells in the medaka, those during migration before hatching have been reported by Gamo [6], while never reported those after hatching when the normal PGCs are located already in the gonad. In the present work, therefore, a preliminary attempt was made to elucidate whether or not the ectopic PGC-like cell in the body wall, though only one cell, has the same ultrastructural features as those of the normal PGCs in the gonad in the same specimen of fry by using electron microscopy.

MATERIALS AND METHODS

The orange-red strain (d-rR) of the Japanese

medaka (*Oryzias latipes*) was used. Under anesthesia in 0.015% phenylurethane aqueous solution, newly hatched fry within a day after hatching were dissected to remove the head and tail. The remaining trunk regions including the gonad were prefixed in a modified Karnovsky's fixative containing 0.1% picric acid [7] for 4–5 hr at room temperature. After rinsing in 0.1 M cacodylate buffer (pH 7.3) at 4°C, they were postfixed in 1% OsO_4 in the same buffer for 1–2 hr at 0°C. The fixed materials were dehydrated in a series of graded ethanol and followed by embedding in a Spurr's resin (Taab). Both thick and thin sections were cut transversely against the body axis of the fry with a diamond knife (Diatome) on a Porter-Blum MT-2B ultratome. The former were stained with toluidine blue for light microscopic observation. The latter were double-stained in uranylacetate and lead nitrate, and examined by a JEM-100 U electron microscopy at 80 kv.

RESULTS AND DISCUSSION

At the time of hatching, most fry had rather small gonads shortly after formed. These gonads were still sexually indifferent and contained a few developmentally undifferentiated large PGCs, each enveloped by thin strands of the somatic cells. In one such specimen of the fry, an ectopic PGC-like cell was seen embedded compactly within a relatively thick subepidermal cell layer just beneath the outermost thin epithelium of the lateral body wall, together with the normal PGCs in the gonad (Figs. 1 and 2A, B). Similar ectopic PGC-like cells could not be detected in another following sections of the remaining parts of the present specimen as far as examined. A basement membrane was evident below the subepidermal layer including the PGC-like cell (Fig. 1, arrowheads and Figs. 2A, B). However, the muscular layer did not develop yet between the parietal and the body wall layers. The PGC-like cell

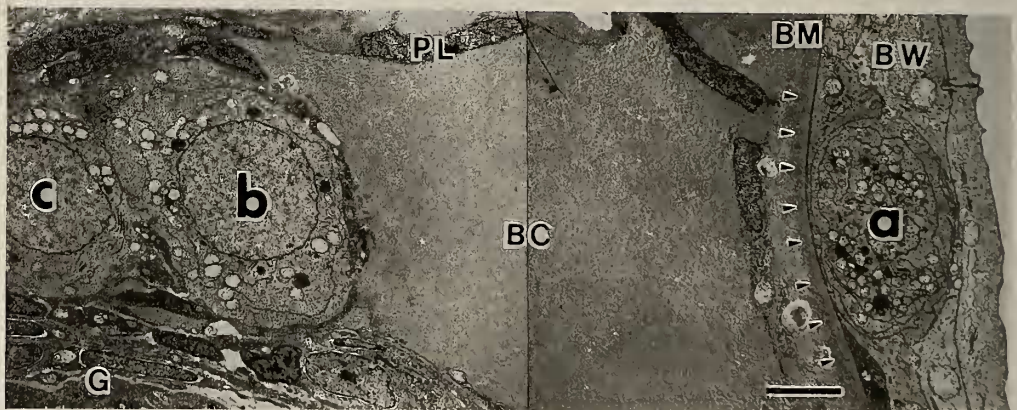


FIG. 1. Low-magnified electron micrograph of a cross section of the posterior trunk region including an ectopic PGC-like cell a in the body wall and two normal PGCs b, c in the sexually indifferent gonad of a newly hatched fry, *Oryzias latipes*. G, gut; BC, body cavity; BW, body wall; PL, parietal layer; BM, basement membrane (arrowheads). Bar, 5 μm .

FIG. 2. A. Electron micrograph of the ectopic PGC-like cell shown as a in Figure 1. B. Another section of the same cell, in which a large, voluminous nucleus with a prominent nucleolus can be seen. Each insertion at the bottom right is a magnification of the encircled area in either A or B, showing the desmosomal connection. Arrowheads indicate occasional, small cytoplasmic protrusions from the cell surface. AR, sheets of agranular reticulum (short arrows); GDB, germinal dense bodies (asterisks); E, epidermal layer; SE, sub-epidermal layer; N, nucleus; Nu, nucleolus; AL, annulate lamella; BM, basement membrane. Long bar, 2 μm . Short bar, 0.2 μm .

FIG. 3. A and B are respectively electron micrographs of two normal PGCs, b and c in the sexually indifferent gonad in Figure 1. SC, enveloping somatic cells; GDB, germinal dense bodies (asterisks); AR, sheets of agranular reticulum (arrows); AL, annulate lamella; BM, basement membrane. Bar, 2 μm .

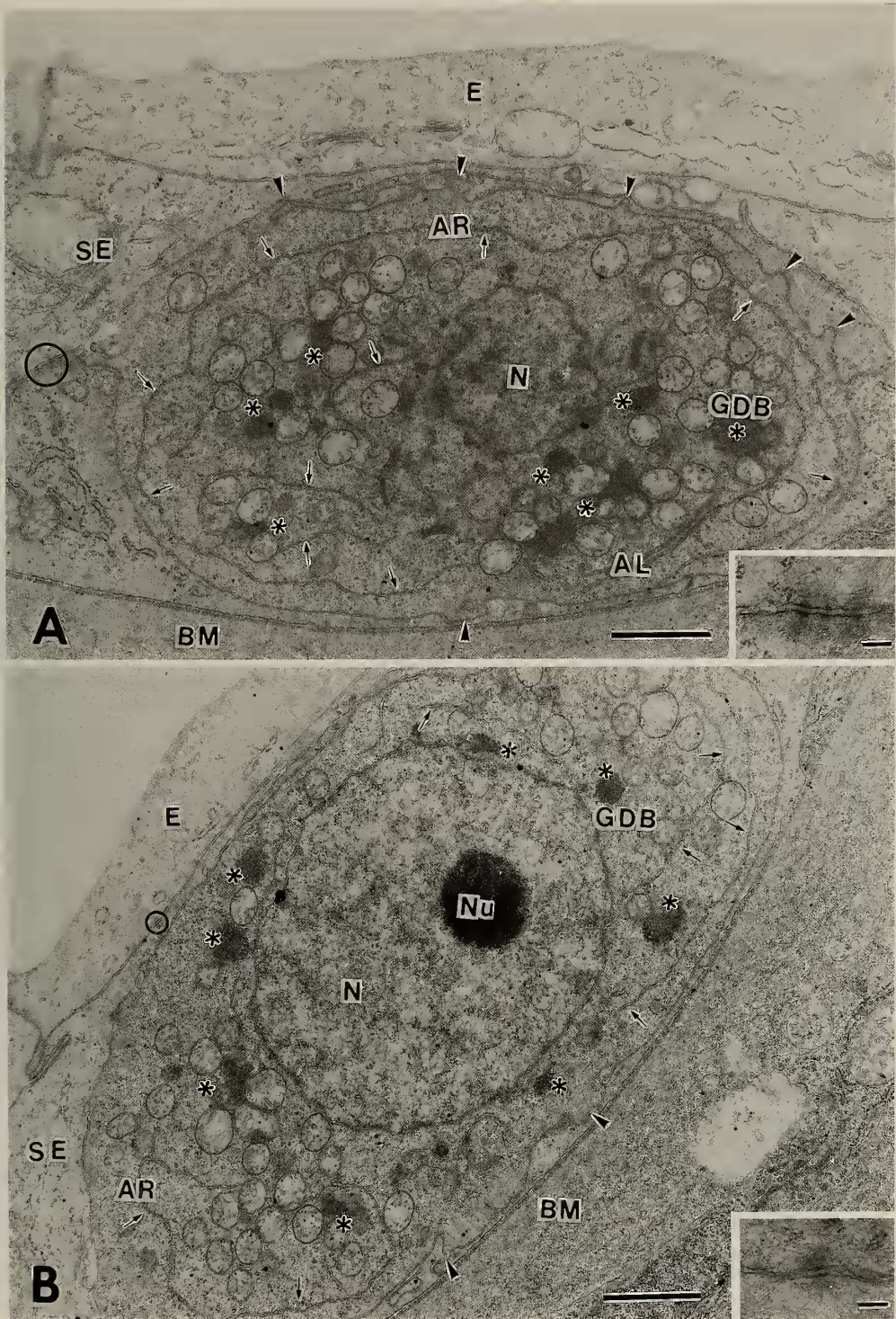


FIG. 2.

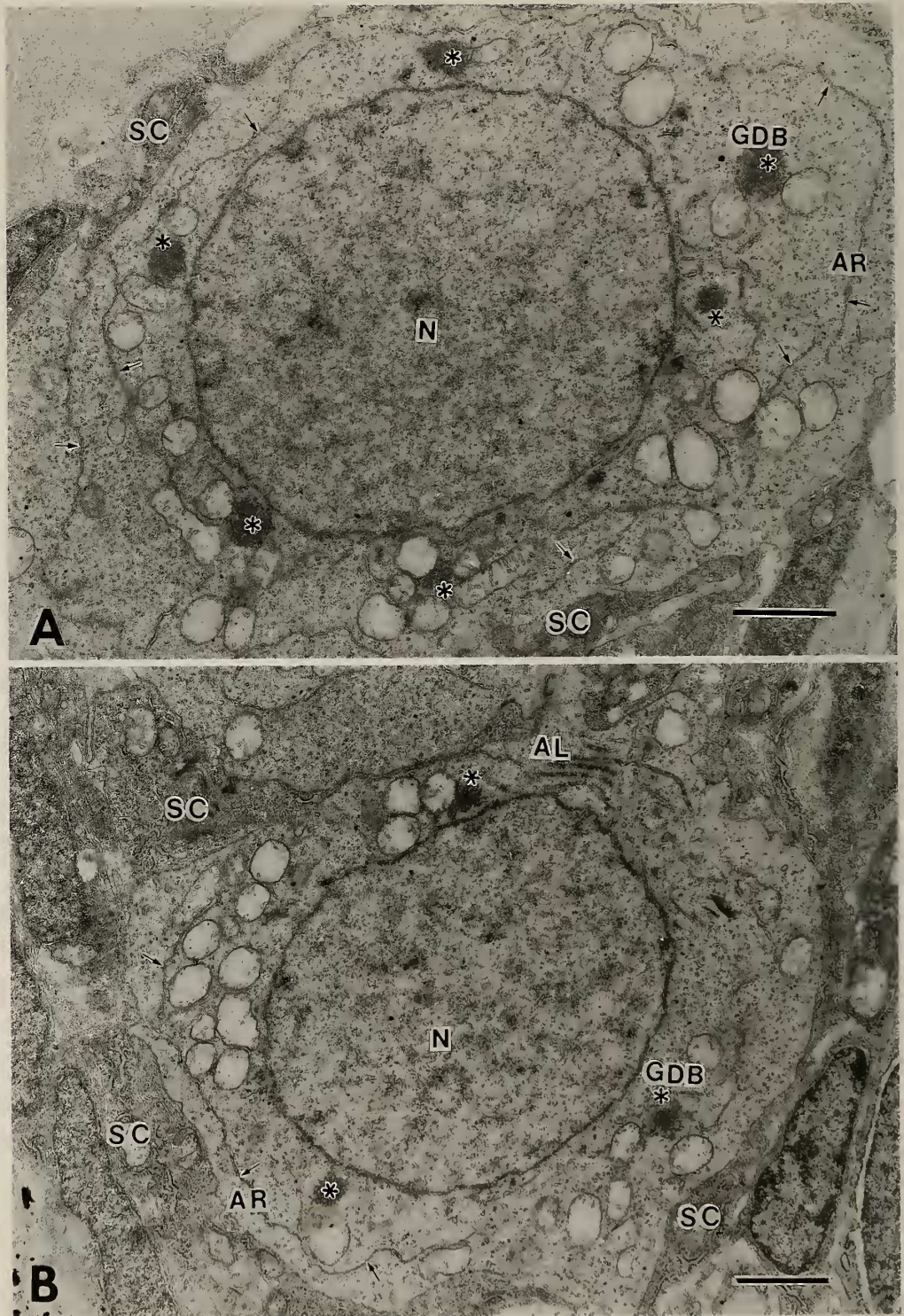


FIG. 3.

appeared somewhat oval in shape, as compared with the normal PGCs in the gonad (Figs. 3A, B), and showed no trace of degenerative appearance. The maximum diameter was about 20 μm and the minimum was about 15 μm , the averaged value of which was near to that of the normal PGCs in the gonad. The nucleus was also large and spherical in outline with a prominent nucleolus (Fig. 2B) as usual for that of the normal gonadal PGCs (not shown). The cytoplasmic membrane of the PGC-like cell was in extensive close contact with that of the surrounding subepidermal cells, but no specific relationship such as desmosomes were observed. In contrast, desmosomes and sometimes anastomosing structures were detectable between the membranes of neighboring cells of either the epidermal or the subepidermal layer, and between those of both layers (Figs. 2A, B., rectangles). On the cell surface of the ectopic PGC-like cell, tiny cytoplasmic processes of various forms were seen protruding into the surrounding cells (Figs. 2A, B, arrowheads). It is uncertain if such pseudopod-like cytoplasmic processes are involved in active migration of the ectopic PGC-like cell during its own amoeboid mobility. In general, passive migration of the PGCs through the morphogenic movement by the surrounding tissues has been proposed in the medaka (*O. latipes*) by Hogan [8] and Hamaguchi [9].

Two characteristic structures that had been first described by Hogan [8] as specific cytological markers for unequivocal identification of the PGCs in the medaka (*O. latipes*) were clearly discernible in the cytoplasm of the ectopic PGC-like cell. One of these was the clumps of germinal dense bodies (GDB) of granular structure sometimes with or without the association of mitochondria (Figs. 2A, B., asterisks). Similar, detailed descriptions of the GDB in the medaka (*O. latipes*) has been presented first by Satoh [10] and later by Hamaguchi [9] and Kanamori *et al.* [11]. The other characteristic structure was the long sheets of agranular reticulum (AR) which often ran in the cytoplasm parallel to the curvature of the nucleus (Figs. 2A, B., short arrows). Occasionally, shorter or loop-forming segments of the sheets were also seen. Unlike such a smooth membraneous structure, poorly stacked, short-linked annulate lamella

(AL) were detectable infrequently. All of these structures, GDB, AR, and AL, could also be observed similarly in the gonadal PGCs (Figs. 3A, B).

As mentioned above, the present observation at the ultrastructural level revealed that the ectopic PGC-like cell in the body wall had almost identical morphological features to those of the normal PGCs in the gonad, principally the two peculiar cytological markers noted above. It may be sure that the present observation is the first demonstration of the ultrastructure of the ectopic-PGC like cell in fish, at least in the medaka.

In the present work, the occurrence of the ectopic PGC-like cell in the mesoderm-derived subepidermal layer of the body wall probably indicates the migratory route rather than the origin of the PGCs. In the medaka, PGCs have been predominantly referred to as having an endodermal origin since they are first recognizable on the peripheral endoderm at the neurula stage, according to Hogan [8] and Hamaguchi [9]. More recently, however, Timmermans and Taverne [12] injected ^3H -thymidine into embryos at an early cleavage stage in the teleost, *Barbus conchoni*, and demonstrated that the presumptive PGCs segregate from the somatic cells between mid epiboly and late epiboly, before the three germ layers have been formed. This result well coincides with that of Gamo's earlier light-microscopical studies in the medaka [12]. Therefore, it is most likely that the ectopic PGC-like cell in the present work is one of the PGCs or their forerunners which has settled down in the extra-gonadal tissues during migration. Moreover, it may be suggested that the presumed forerunners of the PGCs in the medaka, which must have segregated from the somatic cells during the early stages of embryogenesis, could differentiate themselves without any degeneration, even in the ectopic site, at least to the stage of becoming the identifiable PGCs with typical features in the gonad at the time of hatching.

Postnatal differentiation of the ectopic PGCs has been reported in lampreys by Hardisty [3], in several species of adult fish by Stolk [4], and in mice by Zamboni and Upadhyay [5]. In all these works, it is very interesting that the ectopic PGCs

were demonstrated to survive for a certain prolonged period while differentiating as oocytes. It remains to be elucidated whether or not such similar phenomenon as above might occur as for the ectopic PGCs in the medaka. For further study, many more examples of the ectopic PGCs in the medaka during or following embryogenesis will be needed.

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